9 Heat-induced changes in milk

9.1 Introduction

In modern dairy technology, milk is almost always subjected to a heat treatment; typical examples are:

Thermization	e.g. 65°C × 15 s
Pasteurization	
LTLT (low temperature, long time)	63°C × 30 min
HTST (high temperature, short time)	72°C × 15 s
Forewarming (for sterilization)	e.g. 90°C × 2–10 min, 120°C × 2 min
Sterilization	
UHT (ultra-high temperature)	130–140°C × 3–5 s
In-container	110–115°C × 10–20 min

The objective of the heat treatment varies with the product being produced. Thermization is generally used to kill temperature-sensitive micro-organisms, e.g. psychrotrophs, and thereby reduce the microflora of milk for low-temperature storage. The primary objective of pasteurization is to kill pathogens but it also reduces the number of non-pathogenic micro-organisms which may cause spoilage, thereby standardizing the milk as a raw material for various products. Many indigenous enzymes, e.g. lipase, are also inactivated, thus contributing to milk stability. Forewarming (preheating) increases the heat stability of milk for subsequent sterilization (as discussed in section 9.7.1). Sterilization renders milk shelf-stable for very long periods, although gelation and flavour changes occur during storage, especially of UHT-sterilized milks.

Although milk is a very complex biological fluid containing complex protein, lipid, carbohydrate, salt, vitamins and enzyme systems in soluble, colloidal or emulsified states, it is a very heat-stable system, which allows it to be subjected to severe heat treatments with relatively minor changes in comparison to other foods if subjected to similar treatments. However, numerous biological, chemical and physico-chemical changes occur in milk during thermal processing which affect its nutritional, organoleptic and/or technological properties. The temperature dependence of these changes

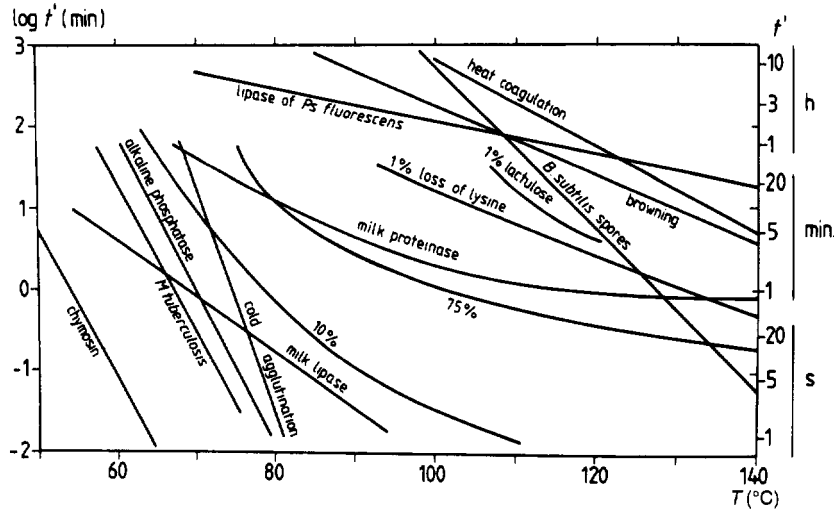


Figure 9.1 The time needed (t') at various temperatures (T) to inactivate some enzymes and cryoglobulins; to kill some bacteria and spores; to cause a certain degree of browning; to convert 1% of lactose to lactulose; to cause heat coagulation; to reduce available lysine by 1%; and to make 10% and 75% of the whey proteins insoluble at pH 4.6 (from Walstra and Jenness, 1984).

Table 9.1 Approximate values for the temperature dependence of some reactions in heated milk (modified from Walstra and Jennes, 1984)

Reaction	Activation energy (kJ mol^{-1})	Q_{10} at 100°C
Many chemical reactions	80–130	2.0–3.0
Many enzyme-catalysed reactions	40–60	1.4–1.7
Autoxidation of lipids	40–100	1.4–2.5
Maillard reactions (browning)	100–180	2.4–5.0
Dephosphorylation of caseinate	110–120	2.6–2.8
Heat coagulation of milk	150	3.7
Degradation of ascorbic acid	60–120	1.7–2.8
Heat denaturation of protein	200–600	6.0–175.0
Typical enzyme inactivation	450	50.0
Inactivation of milk proteinase (plasmin)	75	1.9
Killing vegetative bacteria	200–600	6.0–175.0
Killing of spores	250–330	9.0–17.0

varies widely, as depicted in general terms in Figure 9.1 and Table 9.1. The most significant of these changes, with the exception of the killing of bacteria, will be discussed below. In general, the effect(s) of heat on the principal constituents of milk will be considered individually, although there are interactions between constituents in many cases.

9.2 Lipids

Of the principal constituents, the lipids are probably the least affected by heat. However, significant changes do occur in milk lipids, especially in their physical properties, during heating.

9.2.1 Physicochemical changes

Creaming. The chemical and physicochemical aspects of the lipids in milk were discussed in Chapter 3. The principal effect of heat treatments on milk lipids is on creaming of the fat globules. As discussed in Chapter 3, the fat in milk exists as globules, 0.1–20 μm in diameter (mean, 3–4 μm). The globules are stabilized by a complex membrane acquired within the secretory cell and during excretion from the cell. Owing to differences in density between the fat and aqueous phases, the globules float to the surface to form a cream layer. In cows' milk, the rate of creaming is far in excess of that predicted by Stokes' law, owing to aggregation of the globules which is promoted by cryoglobulins (a group of immunoglobulins). Buffalo, ovine or caprine milks do not undergo cryoglobulin-dependent agglutination of fat globules and cream very slowly with the formation of a compact cream layer.

When milk is heated to a moderate temperature (e.g. 70°C × 15 min), the cryoglobulins are irreversibly denatured and hence the creaming of milk is impaired or prevented; HTST pasteurization (72°C × 15 s) has little or no effect on creaming potential but slightly more severe conditions have an adverse effect (Figure 9.2).

Homogenization, which reduces mean globule diameter to below 1 μm, retards creaming due to the reduction in globule size but, more importantly, to the denaturation of cryoglobulins which prevents agglutination. In fact, there are probably two classes of cryoglobulin, one of which is denatured by heating, the other by homogenization.

Changes in the fat globule membrane. The milk fat globule membrane (MFGM) itself is altered during thermal processing. Milk is usually agitated during heating, perhaps with foam formation. Agitation, especially of warm milk in which the fat is liquid, may cause changes in globule size due to disruption or coalescence; significant disruption occurs during direct UHT processing. Foaming probably causes desorption of some membrane material and its replacement by adsorption of skim-milk proteins. In these cases, it may not be possible to differentiate the effect of heating from the total effect of the process.

Heating *per se* to above 70°C denatures membrane proteins, with the exposure and activation of various amino acid residues, especially cysteine.

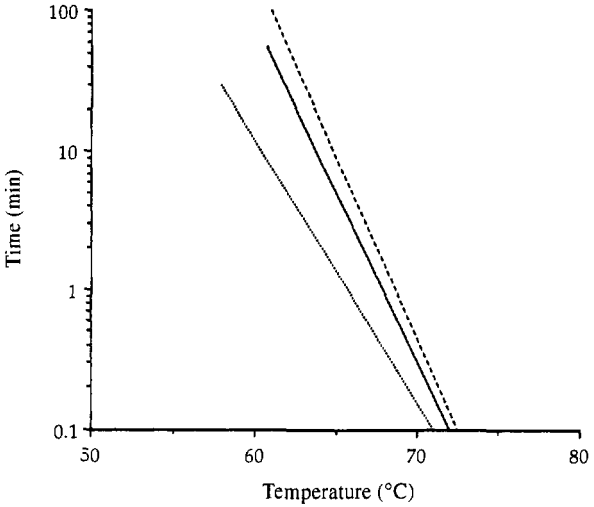


Figure 9.2 Time-temperature curves for the destruction of *M. tuberculosis* (...), inactivation of alkaline phosphatase (_) and creaming ability of milk (– – –) (from Webb and Johnson, 1965).

This may cause the release of H_2S (which can result in the development of an off-flavour) and disulphide interchange reactions with whey proteins, leading to the formation of a layer of denatured whey proteins on the fat globules at high temperatures (>100°C). The membrane and/or whey proteins may participate in Maillard browning with lactose and the cysteine may undergo β-elimination to dehydroalanine, which may then react with lysine to form lysinoalanine or with cysteine residues to form lanthionine, leading to covalent cross-linking of protein molecules (section 9.6.3). Membrane constituents, both proteins and phospholipids, are lost from the membrane to the aqueous phase at high temperatures. Much of the indigenous copper in milk is associated with the MFGM and some of it is transferred to the serum on heat processing. Thus, severe heat treatment of cream improves the oxidative stability of butter made from it as a result of the reduced concentration of pro-oxidant Cu in the fat phase and the antioxidant effect of exposed sulphydryl groups.

The consequences of these changes in the MFGM have been the subject of little study, possibly because severely heated milk products are usually homogenized and an artificial membrane, consisting mainly of casein and some whey proteins, is formed; consequently, changes in the natural membrane are not important. Damage to the membrane of unhomogenized products leads to the formation of free (non-globular) fat and consequently to 'oiling-off' and the formation of a 'cream plug' (Chapter 3).

Severe heat treatment, as is encountered during roller drying and to a lesser extent spray drying, results in at least some demulsification of milk fat, with the formation of free fat, which causes (Chapter 3):

- the appearance of fat droplets when such products are used in tea or coffee;
- increased susceptibility of the fat to oxidation, since it is not protected by a membrane;
- reduced wettability/dispersibility of the powder;
- a tendency of powders to clump.

9.2.2 *Chemical changes*

Severe heat treatments, e.g. frying, may convert hydroxyacids to lactones, which have strong, desirable flavours and contribute to the desirable attributes of milk fat in cooking.

Release of fatty acids and some interesterification may also occur, but such changes are unlikely during the normal processing of milk.

Naturally occurring polyunsaturated fatty acids are methylene-interrupted but may be converted to conjugated isomers at high temperatures. Four

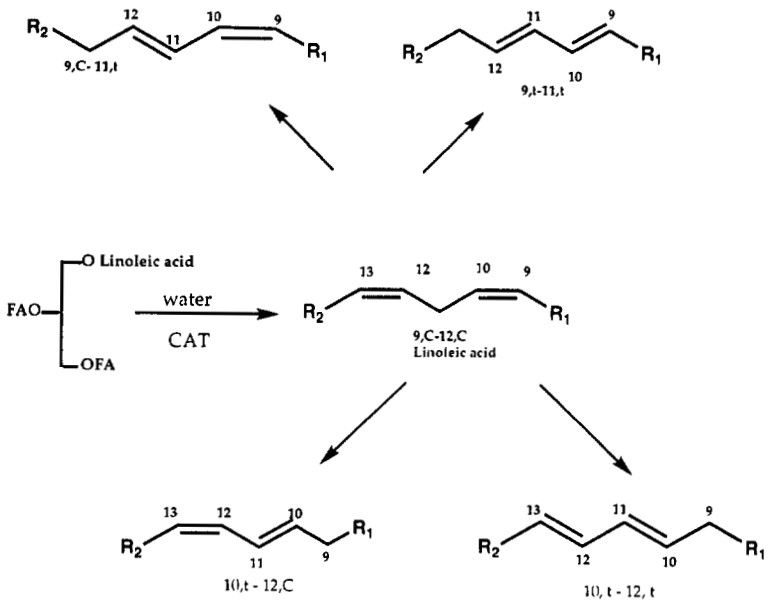


Figure 9.3 Isomers of conjugated linoleic acid.

Table 9.2 Concentration of conjugated linoleic acid (CLA) isomers in selected foods (modified from Ha, Grimm and Pariza, 1989)

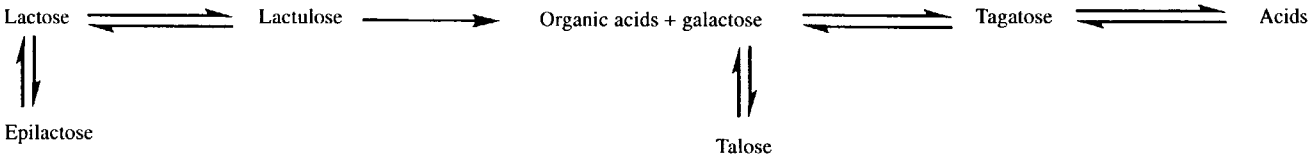
Sample	mg CLA/kg food	Fat content (%)	CLA in fat (mg kg^{-1})
Parmesan cheese	622.3 ± 15.0	32.3 ± 0.9	1926.7
Cheddar cheese	440.6 ± 14.5	32.5 ± 1.7	1355.7
Romano cheese	356.9 ± 6.3	32.1 ± 0.8	1111.9
Blue cheese	169.3 ± 8.9	30.8 ± 1.5	549.8
Processed cheese	574.1 ± 24.8	31.8 ± 1.1	1805.3
Cream cheese	334.5 ± 13.3	35.5 ± 1.0	942.3
Blue spread	202.6 ± 6.1	20.2 ± 0.8	1003.0
Cheese whiz	1815.0 ± 90.3	20.6 ± 1.1	8810.7
Milk			
pasteurized whole	28.3 ± 1.9	4.0 ± 0.3	707.5
non-pasteurized whole	34.0 ± 1.0	4.1 ± 0.1	829.3
Ground beef			
grilled	994.0 ± 30.9	10.7 ± 0.3	9289.7
uncooked	561.7 ± 22.0	27.4 ± 0.2	2050.0

isomers of conjugated linoleic acid (CLA) are shown in Figure 9.3. It is claimed that CLA has anticarcinogenic properties. The mechanism of CLA formation in foods in general is not clear but heat treatment, free radical-type oxidation and microbial enzymatic reactions involving linoleic and linolenic acids in the rumen are thought to be major contributors. Rather high concentrations of CLA have been found in heated dairy products, especially processed cheese (Table 9.2). It has been suggested that whey proteins catalyse isomerization.

9.3 Lactose

The chemistry and physicochemical properties of lactose, a reducing disaccharide containing galactose and glucose linked by a β(1-4)-bond, were described in Chapter 2.

When severely heated in the solid or molten state, lactose, like other sugars, undergoes numerous changes, including mutarotation, various isomerizations and the formation of numerous volatile compounds, including acids, furfural, hydroxymethylfurfural, CO_2 and CO. In solution under strongly acidic conditions, lactose is degraded on heating to monosaccharides and other products, including acids. These changes do not normally occur during the thermal processing of milk. However, lactose is relatively unstable under mild alkaline conditions at moderate temperatures where it undergoes the Lobry de Bruyn–Alberda van Ekenstein rearrangement of aldoses to ketoses (Figure 9.4).



[Epilactose = 4-O-β-D-galactopyranosyl-D-mannopyranose
Lactulose = 4-O-β-D-galactopysanosyl-D-fructofuranose]

Figure 9.4 Heat-induced changes in lactose under mild alkaline conditions.

Lactose undergoes at least three heat-induced changes during the processing and storage of milk and milk products.

9.3.1 Formation of lactulose

On heating at low temperatures under slightly alkaline conditions, the glucose moiety of lactose is epimerized to fructose with the formation of lactulose, which does not occur in nature. The significance of lactulose has been discussed in Chapter 2. Lactulose is not formed during HTST processing but is formed during UHT sterilization (more during indirect than direct heating) and especially during in-container sterilization; therefore, the concentration of lactulose in milk is a useful index of the severity of the heat treatment to which the milk has been subjected (see Figure 2.19). The concentration of lactulose is probably the best index available at present for differentiating between UHT and in-container sterilized milks and a number of assay procedures have been developed, using HPLC or enzymatic/spectrophotometric principles.

9.3.2 Formation of acids

Milk as secreted by the cow contains about 200 mg CO_2 l^{-1}. Owing to its low concentration in air, CO_2 is rapidly and, in effect, irreversibly lost from milk on standing after milking; its loss is accelerated by heating, agitation

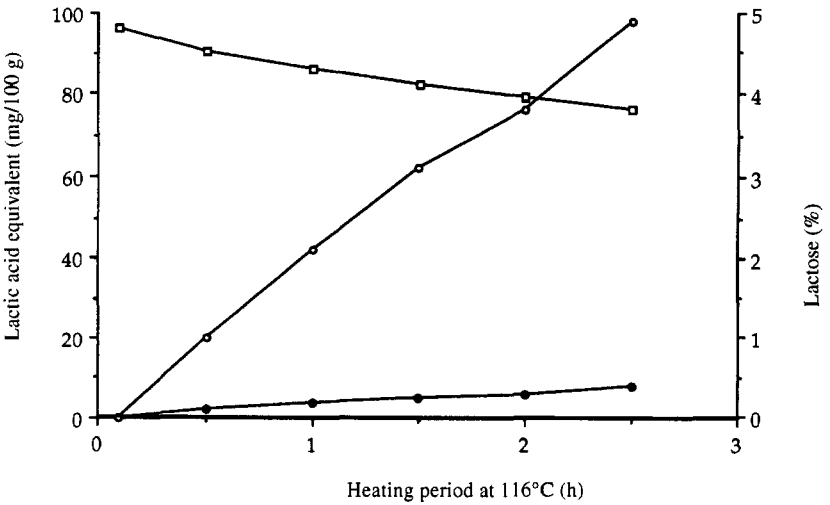


Figure 9.5 Changes in titratable acidity (○), lactic acid (●) and lactose (□) on heating homogenized milk in sealed cans at 116°C. Titratable acidity expressed as mg lactic acid/100 g milk (from Gould, 1945.)

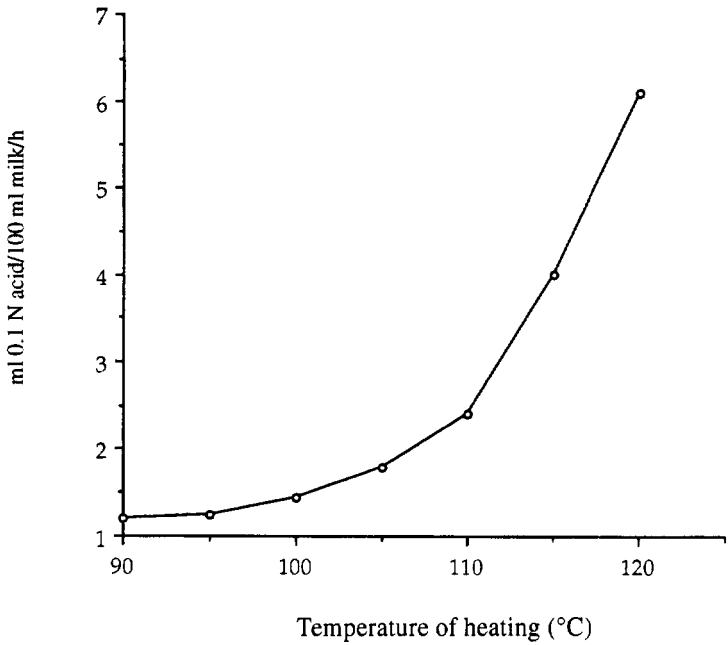


Figure 9.6 Effect of temperature on the rate of heat-induced production of acid in milk (from Jenness and Patton, 1959).

and vacuum treatment. This loss of CO_2 causes an increase in pH of about 0.1 unit and a decrease in the titratable acidity of nearly 0.02%, expressed as lactic acid. Under relatively mild heating conditions, this change in pH is more or less offset by the release of H^+ on precipitation of $Ca_3(PO_4)_2$, as discussed in section 9.4.

On heating at temperatures above 100°C, lactose is degraded to acids with a concomitant increase in titratable acidity (Figures 9.5, 9.6). Formic acid is the principal acid formed; lactic acid represents only about 5% of the acids formed. Acid production is significant in the heat stability of milk, e.g. when assayed at 130°C, the pH falls to about 5.8 at the point of coagulation (after about 20 min) (Figure 9.7). About half of this decrease is due to the formation of organic acids from lactose; the remainder is due to the precipitation of calcium phosphate and dephosphorylation of casein, as discussed in section 9.4.

In-container sterilization of milk at 115°C causes the pH to decrease to about 6 but much of this is due to the precipitation of calcium phosphate; the contribution of acids derived from lactose has not been quantified accurately. Other commercial heat treatments, including UHT sterilization, cause insignificant degradation of lactose to acids.

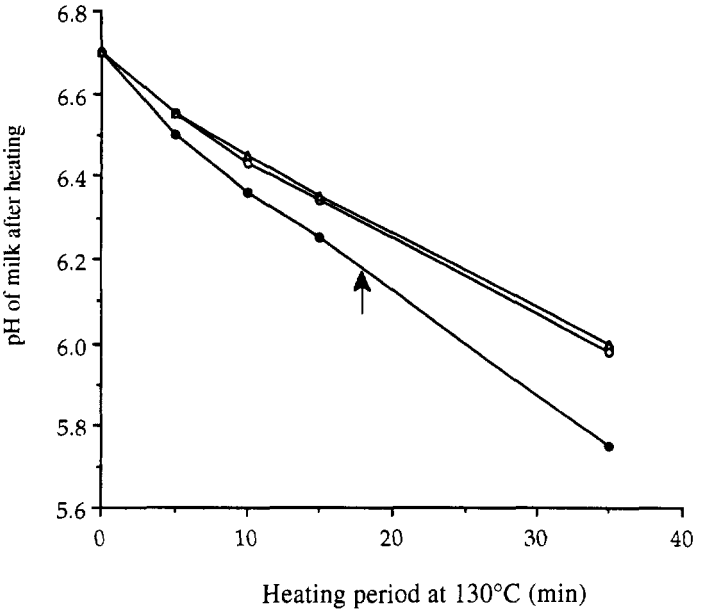


Figure 9.7 The pH of samples of milk after heating for various periods at 130°C with air (○), O_2 (●) or N_2 (△) in the headspace above the milk; ↑, coagulation time (from Sweetsur and White, 1975).

9.3.3 Maillard browning

The mechanism and consequences of the Maillard reaction were discussed in Chapter 2. The reaction is most significant in severely heat-treated products, especially in-container sterilized milks. However, it may also occur to a significant extent in milk powders stored under conditions of high humidity and high temperature, resulting in a decrease in the solubility of the powder. If cheese contains a high level of residual lactose or galactose (due to the use of a starter unable to utilize galactose; Chapter 10), it is susceptible to Maillard browning, especially during cooking on pizza, e.g. Mozzarella (Pizza) cheese. Browning may also occur in grated cheese during storage if the cheese contains residual sugars; in this case, the water activity of the cheese ($a_w \sim 0.6$) is favourable for the Maillard reaction. Poorly washed casein and especially whey protein concentrates (which contain 30–60% lactose) may undergo Maillard browning when used as ingredients in heat-treated foods.

Maillard browning in milk products is undesirable because:

1. The final polymerization products (melanoidins) are brown and hence dairy products which have undergone Maillard browning are discoloured and aesthetically unacceptable.

2. Some of the by-products of Maillard browning have strong flavours (e.g. furfural, hydroxymethylfurfural) which alter the typical flavour of milk.
3. The initial Schiff base is digestible but after the Amadori rearrangement, the products are not metabolically available. Since lysine is the amino acid most likely to be involved and is an essential amino acid, Maillard browning reduces the biological value of proteins. Interaction of lysine with lactose renders the adjacent peptide bond resistant to hydrolysis by trypsin, thereby reducing the digestibility of the protein.
4. The polymerized products of Maillard browning can bind metals, especially Fe.
5. It has been suggested that some products of the Maillard reaction are toxic and/or mutagenic but such effects are, at most, weak and possibly due to other consequences of browning, e.g. metal binding.
6. The attachment of sugars to the protein increases its hydrophilicity; however, solubility may be reduced, probably due to cross-linking of protein molecules.
7. The heat stability of milk is increased by the Maillard reaction, probably via the production of carbonyls (section 9.7).

The formation of brown pigments via the Maillard reaction, especially in model systems (e.g. glucose–glycine), usually follows zero-order kinetics, but the loss of reactants has been found to follow first- or second-order kinetics in foods and model systems. Activation energies of 109, 116 and 139 kJ mol^{-1} have been reported for the degradation of lysine, the formation of brown pigments and the production of hydroxymethylfurfural (HMF), respectively.

Browning can be monitored by measuring the intensity of brown colour, the formation of hydroxymethylfurfural (which may be measured spectrophotometrically, after reaction with thiobarbituric acid, or by HPLC, but which is not regarded as a very good indicator of Maillard browning), loss of available lysine (e.g. by reaction with 2,4-dinitrofluorobenzene) or by the formation of furosine. Furosine is formed on the acid hydrolysis of lactulosyl lysine (the principal Maillard product formed during the heating of milk). During acid hydrolysis, lactulosyl lysine is degraded to fructosylysine which is then converted to pyridosine, furosine and carboxymethyl lysine (Figure 9.8). Furosine may be determined by ion-exchange chromatography, GC or HPLC, and is considered to be a very good indicator of Maillard browning and the severity of heat treatment of milk (Erbersdobler and Dehn-Müller, 1989). The effects of time and temperature on the formation of furosine are shown in Figure 9.9. The concentration of furosine is highly correlated with the concentrations of HMF and carboxymethyl lysine. The concentration of furosine in commercial UHT milks is shown in Figure 9.10.

Dicarbonyls, which are among the products of the Maillard reaction, can react with amines in the Strecker reaction, producing a variety of flavourful

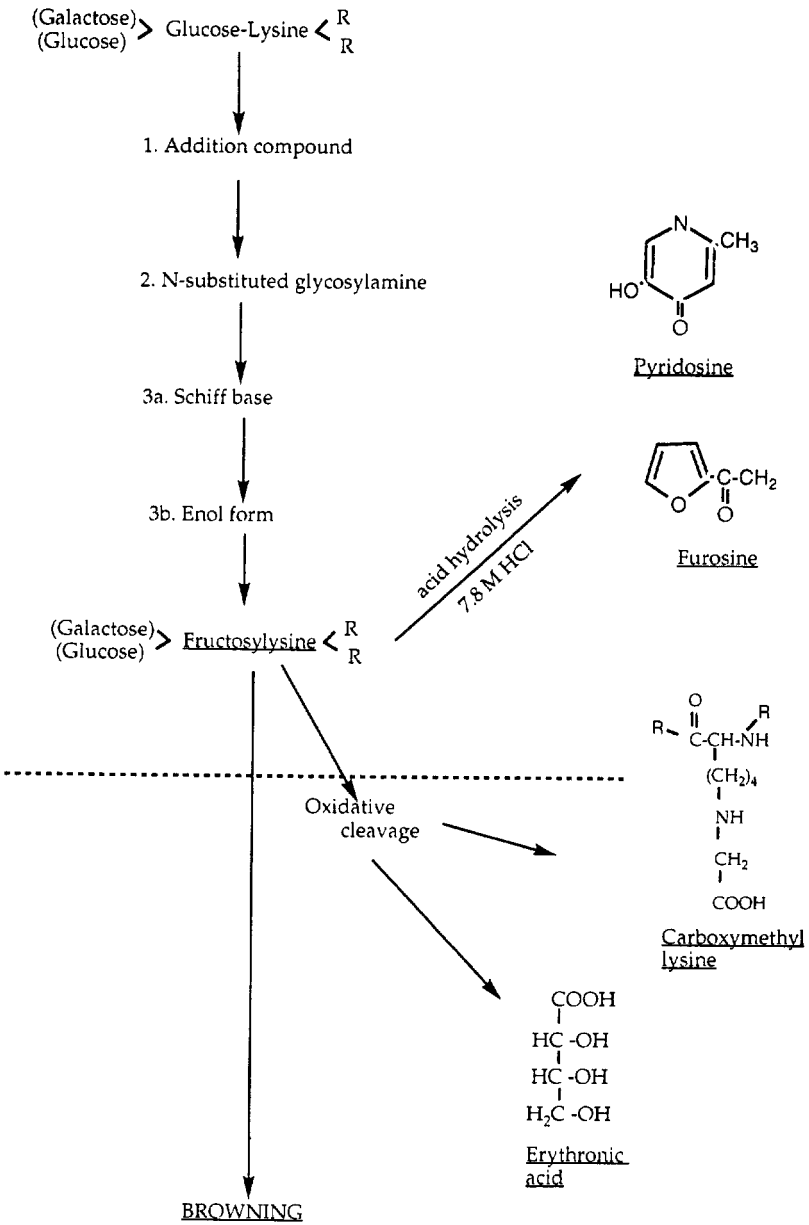


Figure 9.8 Initial steps of the Maillard reaction with the formation of furosine (after hydrolysis with 7.8 M HCl) as well as of *N*-ε-carboxymethyl lysine and erythronic acid (from Erbersdobler and Dehn-Müller, 1989).

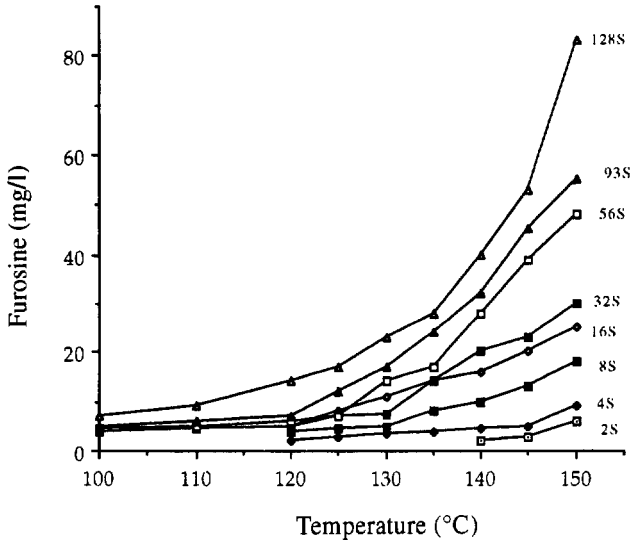


Figure 9.9 Effect of heating temperature and time on the concentration of furosine in directly heated UHT milks (from Erbersdobler and Dehn-Müller, 1989).

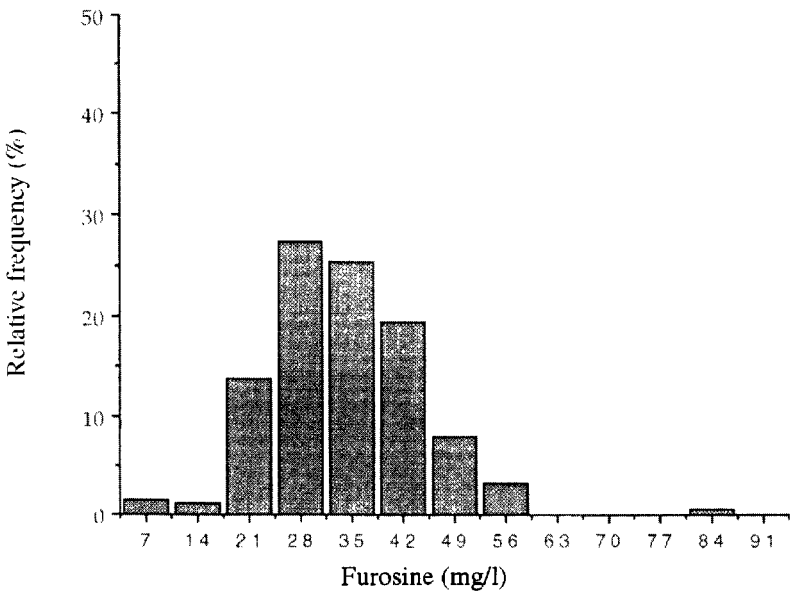


Figure 9.10 Relative distribution of the furosine concentrations in 190 commercial UHT milks in increments of 7 mg furosine (from Erbersdobler and Dehn-Müller, 1989).

compounds (Figure 2.32). The Maillard and especially the Strecker reactions can occur in cheese and may be significant contributors to flavour; in this case, the dicarbonyls are probably produced via biological, rather than thermal, reactions.

9.4 Milk salts

Although the organic and inorganic salts of milk are relatively minor constituents in quantitative terms, they have major effects on many aspects of milk, as discussed in Chapter 5. Heating has little effect on milk salts with two exceptions, carbonates and calcium phosphates. Most of the potential carbonate occurs as CO_2 which is lost on heating, with a consequent increase in pH. Among the salts of milk, calcium phosphate is unique in that its solubility decreases with increasing temperature. On heating, soluble calcium phosphate precipitates on to the casein micelles, with a concomitant decrease in the concentration of calcium ions and pH (Chapter 5). These changes are reversible on cooling if the heat treatment was not severe. Following severe heat treatment, the heat-precipitated calcium phosphate is probably insoluble but some indigenous colloidal calcium phosphate dissolves on cooling to partly restore the pH. The situation becomes rather complex in severely heated milk due to the decrease in pH caused by thermal degradation of lactose and dephosphorylation of casein.

The cooling and freezing of milk also cause shifts in the salts equilibria in milk, including changes in pH, as discussed in Chapters 2, 5 and 11.

9.5 Vitamins

Many of the vitamins in milk are relatively heat labile, as discussed in Chapter 6.

9.6 Proteins

The proteins of milk are probably the constituents most affected by heating. Some of the changes involve interaction with salts or sugars and, although not always fully independent of changes in other constituents, the principal heat-induced changes in proteins are discussed in this section.

9.6.1 Enzymes

As discussed in Chapter 8, milk contains about 60 indigenous enzymes derived from the secretory cells or from blood. Stored milk may also contain enzymes produced by micro-organisms. Both indigenous and bacterial

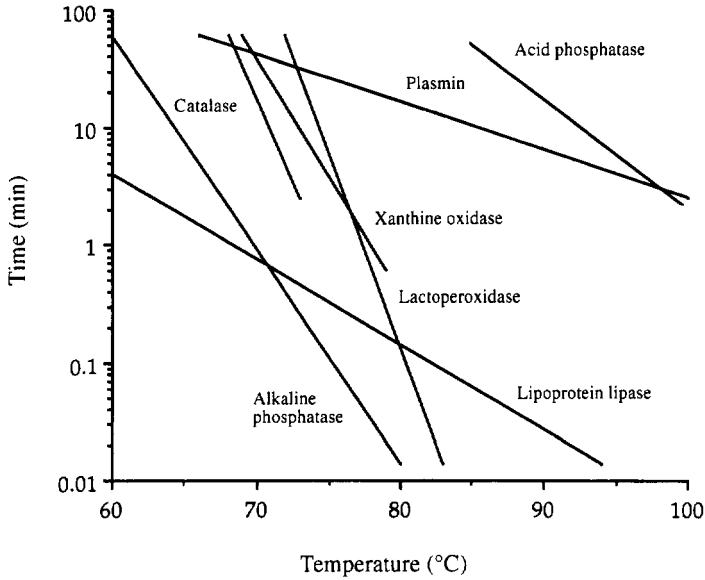


Figure 9.11 Time-temperature combinations required for which milk must be heated to a certain temperature to inactivate some indigenous milk enzymes (from Walstra and Jenness, 1984).

enzymes can have undesirable effects in milk and dairy products. Although not the primary objective of thermal processing, some of the indigenous enzymes in milk are inactivated by the commercially used heat processes, although many are relatively heat stable (Figure 9.11).

The thermal denaturation of indigenous milk enzymes is important from two major viewpoints:

1. To increase the stability of milk products. Lipoprotein lipase is probably the most important in this regard as its activity leads to hydrolytic rancidity. It is extensively inactivated by HTST pasteurization but heating at 78°C × 10 s is required to prevent lipolysis. Plasmin activity is actually increased by HTST pasteurization due to inactivation of inhibitors of plasmin and/or of plasminogen activators.
2. The activity of selected enzymes is used as indices of thermal treatments, e.g. alkaline phosphatase (HTST pasteurization), γ-glutamyl transpeptidase (index of heating in the range 72–80°C) or lactoperoxidase (80–90°C).

Microbial enzymes. The widespread use of refrigerated storage of milk at farm and factory for extended periods has led to psychrotrophs, especially

Pseudomonas fluorescens, becoming the dominant micro-organisms in raw milk supplies. Psychrotrophs are quite heat labile and are readily killed by HTST pasteurization and even by thermization. However, they secrete extracellular proteinases, lipases and phospholipases that are extremely heat stable – some are not completely inactivated by heating at 140°C for 1 min and thus partially survive UHT processing. If the raw milk supply contains high numbers of psychrotrophs ($>10^6$ per ml), the amounts of proteinase and lipase that survive UHT processing may be sufficient to cause off-flavours, such as bitterness, unclean and rancid flavours, and perhaps gelation.

One of the very curious characteristics of the proteinases and lipases secreted by many psychrotrophs is that they have relatively low stability in the temperature range 50–65°C, Figure 9.12 (the precise value depends on the enzyme). Thus, it is possible to reduce the activity of these enzymes in milk by a low temperature inactivation (LTI) treatment (e.g. 60°C × 5–10 min) before or after UHT processing. Inactivation of the proteinase by LTI appears to be due mainly to proteolysis; in the native state, the enzyme is tightly folded and resistant to proteolysis by other proteinase molecules in its neighbourhood but at about 60°C, some molecules undergo conformational changes, rendering them susceptible to proteolysis by proteinase molecules which are still active. On increasing the temperature further, all proteinase molecules are denatured and inactive but they can renature on

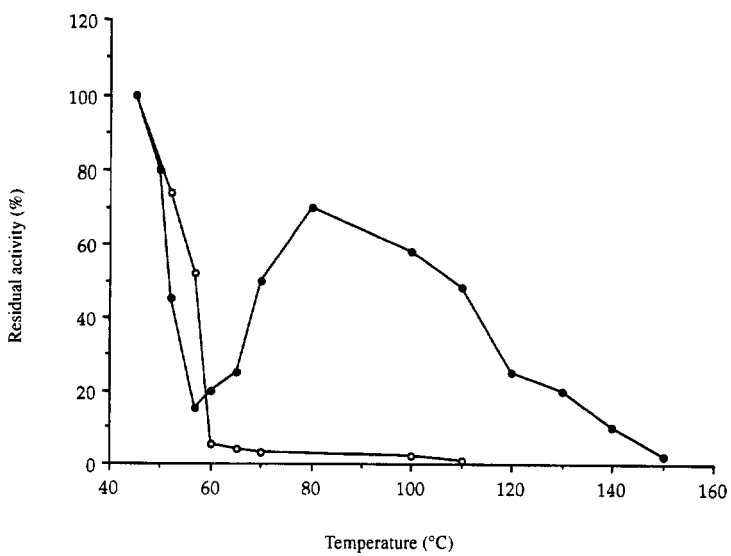


Figure 9.12 Thermal inactivation of *Ps. fluorescens* AFT 36 proteinase on heating for 1 min in 0.1 M phosphate buffer, pH 6.6 (○) or in a synthetic milk salts buffer, pH 6 (●) (from Stepaniak, Fox and Daly, 1982).

cooling. Since this mechanism does not apply to purified lipase, the mechanism of LTI of lipase is not clear (for reviews on enzymes from psychrotrophs see Driessen (1989) and McKellar (1989)).

9.6.2 Denaturation of other biologically active proteins

Milk contains a range of biologically active proteins, e.g. vitamin-binding proteins, immunoglobulins, metal-binding proteins, antibacterial proteins (lactotransferrin, lysozyme, lactoperoxidase), various growth factors and hormones (Chapters 4 and 8). These proteins play important nutritional and physiological functions in the neonate. All these proteins are relatively heat labile – some are inactivated by HTST pasteurization and probably all are inactivated by UHT and more severe heat treatments. Inactivation of these biologically active proteins may not be particularly important when milk is used in the diet of adults but may be highly significant in infant formulae; consequently, supplementation of infant formulae with some of these proteins is advocated.

9.6.3 Denaturation of whey proteins

The whey proteins, which represent about 20% of the proteins of bovine milk, are typical globular proteins with high levels of secondary and tertiary structures, and are, therefore, susceptible to denaturation by various agents, including heat. The denaturation kinetics of whey proteins, as measured by loss of solubility in saturated NaCl at pH 4.6, are summarized in Figure

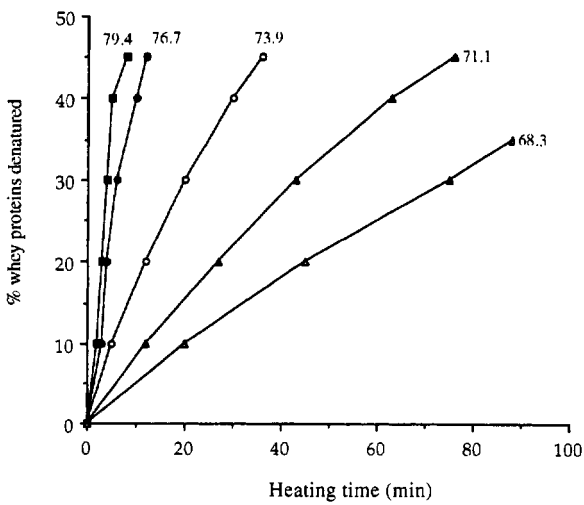


Figure 9.13 Heat denaturation of whey proteins on heating skim milk at various temperatures (°C) as measured by precipitability with saturated NaCl (from Jenness and Patton, 1959).

9.13. Thermal denaturation is a traditional method for the recovery of proteins from whey as 'lactalbumin'; coagulation is optimal at pH 6 and about 90°C for 10 min (Chapter 4).

The order of heat stability of the whey proteins, measured by loss of solubility, is: α-lactalbumin (α-la) > β-lactoglobulin (β-lg) > blood serum albumin (BSA) > immunoglobulins (Ig) (Figure 9.14). However, when measured by differential scanning calorimetry, quite a different order is observed: Ig > β-lg > α-la > BSA. In the case of α-la, the discrepancy appears to be explained by the fact that it is a metallo (Ca)-protein which renatures quite readily following thermal denaturation. However, the Ca-free apoprotein is quite heat labile, a fact which is exploited in the isolation of α-la. The Ca^{2+} is bound in a pocket to the carboxylic acid groups of three Asp residues and the carbonyls of an Asp and a Lys residue (Chapter 4). The carboxylic acid groups become protonated below about pH 5 and lose their ability to bind Ca; the apoprotein can be aggregated by heating to about 55°C, leaving mainly β-lg in solution. Apo-lactoferrin is also considerably less stable than the intact protein.

The denaturation of α-la and β-lg in milk follows first- and second-order kinetics, respectively (Figure 9.15). Both proteins show a change in the temperature-dependence of denaturation at about 90°C (Figure 9.15).

The mechanism of the thermal denaturation of β-lg has been studied extensively; the sequence of events is shown schematically in Figure 9.16. At about 20°C in the pH range 5.5–7.0, β-lg exists as an equilibrium between

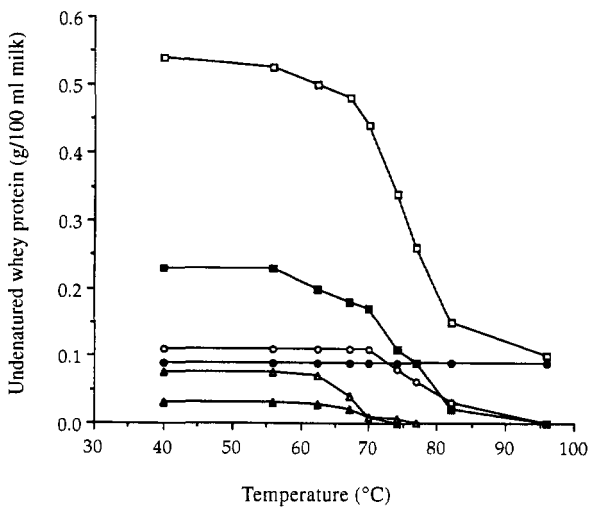


Figure 9.14 The denaturation of the total (□) and individual whey proteins in milk, heated at various temperatures for 30 min; β-lactoglobulin (■), α-lactalbumin (○), proteose peptone (●), immunoglobulins (△), and serum albumin (▲) (from Webb and Johnson, 1965).

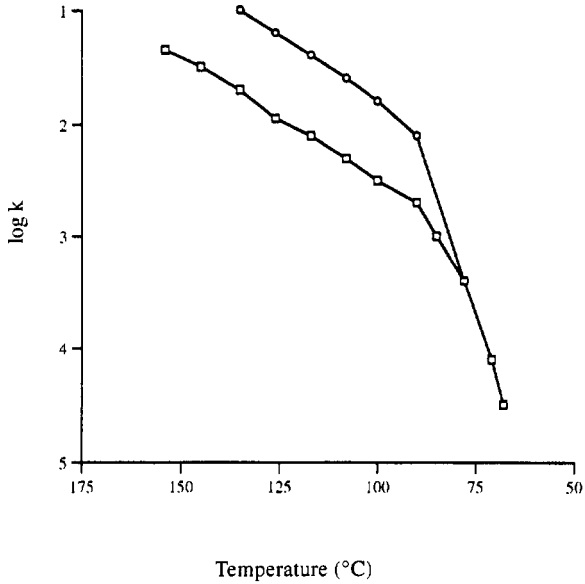


Figure 9.15 Arrhenius plot of the rate constant for the heat treatment of α-lactalbumin (□) and β-lactoglobulin (○) (from Lyster, 1970).

its dimeric (N_2) and monomeric (2N) forms. Between pH 7 and 9, it undergoes a reversible conformational change, referred to as the $N \rightleftarrows R$ transition. Both equilibria are pushed to the right as the temperature is increased, i.e. $N_2 \rightarrow 2N \rightarrow 2R$. Above about 65°C, β-lg undergoes reversible denaturation ($R \rightleftarrows D$) but at about 70°C, denaturation becomes irreversible via a series of aggregation steps. The initial type I aggregation involves the formation of intermolecular disulphide bonds while the later type II aggregation involves non-specific interactions, including hydrophobic and electrostatic bonding. Type III aggregation involves non-specific interactions and occurs when the sulphydryl groups are blocked.

Some of the most important consequences of the heat denaturation of whey proteins are due to the fact that these proteins contain sulphydryl and/or disulphide residues which are exposed on heating (Figure 9.17). They are important for at least the following reasons:

1. The proteins can participate in sulphydryl–disulphide interchange reactions at temperatures above about 75°C at the pH of milk, but more rapidly at or above pH 7.5. Such interactions lead to the formation of disulphide-linked complexes of β-lg with κ-casein, and probably α_{s2}-casein and α-la, with profound effects on the functionality of the milk protein system, such as rennet coagulation and heat stability.

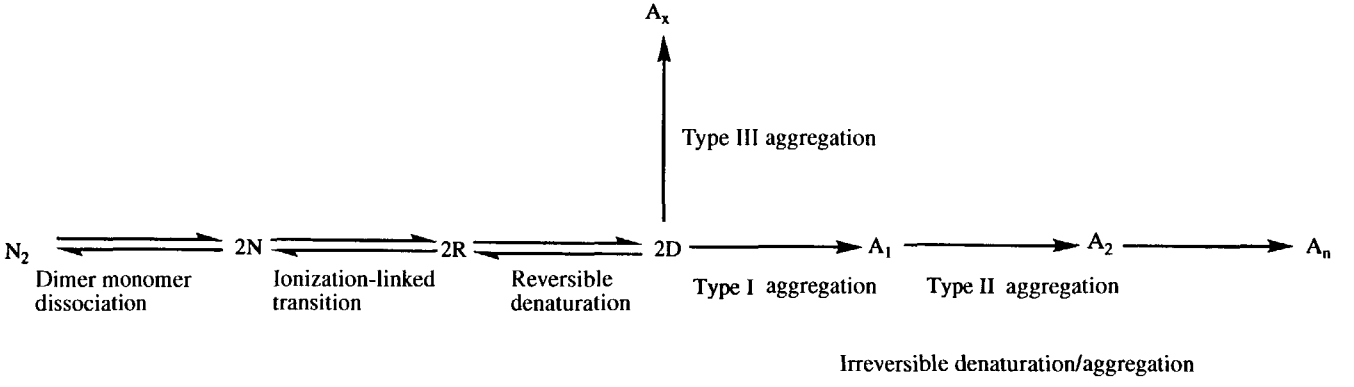


Figure 9.16 Stages in the thermal denaturation of β-lactoglobulin (from Mulvihill and Donovan, 1987).

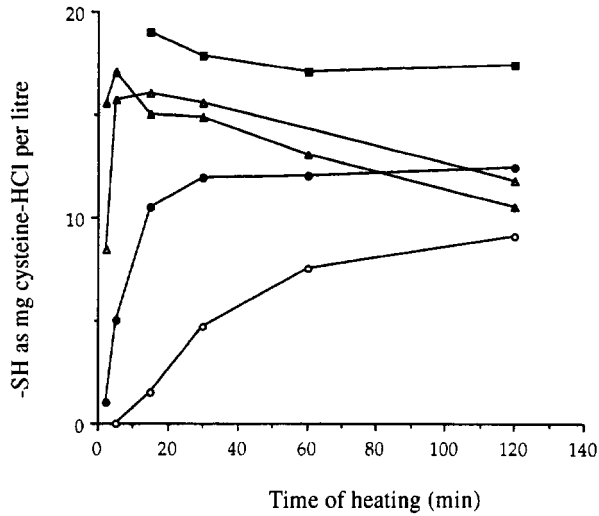


Figure 9.17 Exposure of sulphydryl groups by heating milk at 75 (○), 80 (●), 85 (△) or 95 (▲) °C; de-aerated milk heated at 85°C (■) (from Jenness and Patton, 1959).

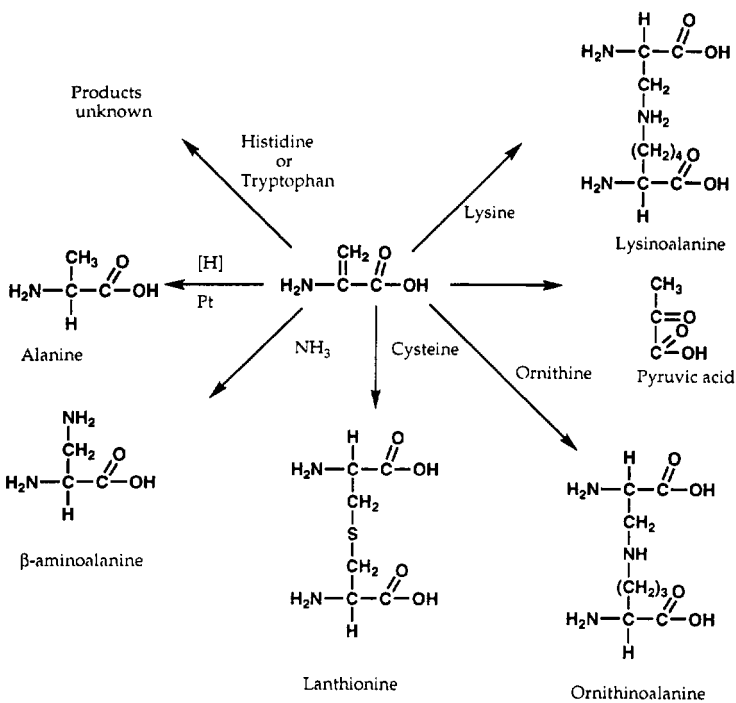


Figure 9.18 Interaction of dehydroalanine with amino acids.

2. The activated sulphydryls may decompose with the formation of H_2S and $H_3C\text{-}S\text{-}CH_3$, which are responsible for the cooked flavour of severely heated milk, including UHT milk. These compounds are volatile and unstable and disappear within about 1 week after processing so that the flavour of UHT milk improves during the first few weeks after processing.
3. Serine, serine phosphate, glycosylated serine, cysteine and cystine residues can undergo β-elimination with the formation of dehydroalanine. Dehydroalanine is very reactive and can react with various amino acid residues, especially lysine, leading to the formation of lysinoalanine, and to a lesser extent with cysteine with the formation of lanthionine (Figure 9.18). These reactions lead to intra- or intermolecular cross-linking which reduce protein solubility, digestibility and nutritive value (because the bonds formed are not hydrolysed in the intestinal tract and lysine is an essential amino acid). Although there are reports to the contrary, lysinoalanine is not normally found in UHT milk or cream.

9.6.4 *Effect of heat on caseins*

As discussed in Chapter 4, the caseins are rather unique proteins. They are rather small (20–25 kDa), relatively hydrophobic molecules, with little higher structure, few disulphide bonds (present only in the two minor caseins, α_{s2} and κ) and no sulphydryl groups. All the caseins are phosphorylated (8–9, 10–13, 4–5 and 1 mole P per mole protein for α_{s1}-, α_{s2}-, β- and κ-casein, respectively); due to their high levels of phosphorylation, α_{s1}-, α_{s2}- and β-caseins bind calcium strongly, causing them to aggregate and precipitate, and affecting their general stability, including heat stability.

Within the strict sense of the term, the caseins are not susceptible to thermal denaturation, e.g. sodium caseinate (pH 6.5–7.0) may be heated at 140°C for more than 1 h without any visible physicochemical changes. However, severe heat treatments do cause substantial changes, e.g. dephosphorylation (about 100% in 1 h at 140°C), aggregation (as indicated by changes in urea–PAGE or gel permeation chromatography), possibly due to the formation of intermolecular disulphide and intermolecular isopeptide bonds, cleavage of peptide bonds (formation of peptides soluble at pH 4.6 or in 12% TCA). β-Elimination of serine, serine phosphate and cysteine residues may also occur, especially at pH values above 7. Such heat-induced changes are evident in commercial sodium caseinate.

The remarkably high heat stability of the caseins allows heat-sterilized dairy products to be produced without major changes in physical properties (reviewed by Fox, 1982; Singh and Creamer, 1992). The heat stability of unconcentrated milk is almost always adequate to withstand the temperature treatments to which it is normally subjected; only rarely is a defect known as the 'Utrecht phenomenon' encountered, when milk coagulates on HTST heating. This defect is due to a very high Ca^{2+} concentration owing

to a low concentration of citrate, arising from poor feed. However, the heat stability of milk decreases sharply on concentration and is usually inadequate to withstand in-container or UHT processing unless certain adjustments and/or treatments are made. Although the heat stability of concentrated milk is poorly correlated with that of the original milk, most of the research on the heat stability of milk has been done on unconcentrated milk.

9.7 Heat stability of milk

Studies on the heat stability of milk date from the pioneering work of Sommer and Hart, which commenced in 1919. Much of the early work concentrated on attempts to relate heat stability to variations in milk composition, especially the concentrations of milk salts. Although the heat coagulation time (HCT) of milk is inversely related to the concentrations of divalent cations (Ca^{2+} and Mg^{2+}) and positively with the concentrations of polyvalent anions (i.e. phosphate and citrate), the correlations are poor and unable to explain the natural variations in HCT. This failure was largely explained in 1961 by Rose who showed that the HCT of most milks is extremely sensitive to small changes in pH in the neighbourhood of 6.7. In effect, the influence of all other factors on the HCT of milk must be considered against the background of the effect of pH.

For the majority of individual-cow and all bulk milks, the HCT increases with increasing pH from 6.4 to about 6.7, then decreases abruptly to a minimum at around pH 6.9 but increases continuously with further increases in pH (Figure 9.19). The HCT decreases sharply below pH 6.4. Milks which show a strong dependence of heat stability on pH are referred to as type A milks. Occasionally, the HCT of individual-cow milks increases continuously with increasing pH, which is as would be expected due to increasing protein charge with increasing pH; these are referred to as type B milks.

The maximum HCT and the shape of the HCT–pH profile are influenced by several compositional factors, of which the following are the most significant:

1. Ca^{2+} reduces HCT throughout the pH range 6.4–7.4.
2. Ca-chelators, e.g. citrate, polyphosphate, increase stability.
3. β-Lg, and probably α-la, increase the stability of casein micelles at pH 6.4–6.7 but reduce it at pH 6.7–7.0; in fact, the occurrence of a maximum–minimum in the HCT–pH profile depends on the presence of β-lg.
4. Addition of κ-casein to milk increases stability in the pH range of the HCT minimum.

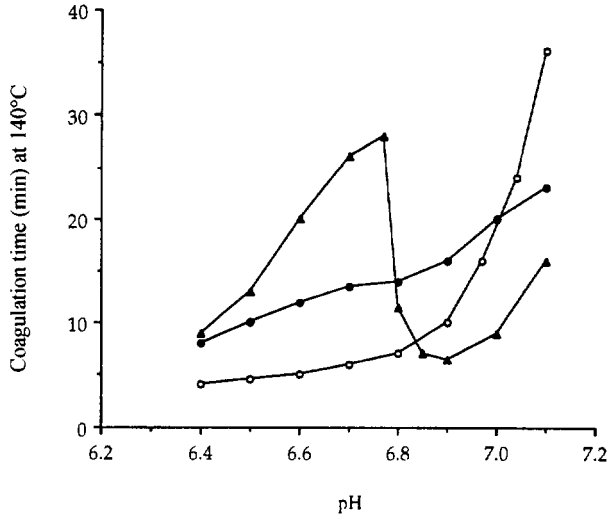


Figure 9.19 Effect of pH on the heat stability of type A milk (▲), type B milk (●) and whey protein-free casein micelle dispersions (○) (from Fox, 1982).

5. Reducing the level of colloidal calcium phosphate increases stability in the region of the HCT maximum.
6. Natural variations in HCT are due mainly to variations in the concentration of indigenous urea due to changes in the animals' feed.

The current explanation for the maximum–minimum in the HCT–pH profile is that on heating, κ-casein dissociates from the micelles; at pH values below about 6.7, β-lg reduces the dissociation of κ-casein, but at pH values above 6.7, it accentuates dissociation. In effect, coagulation in the pH range of minimum stability involves aggregation of κ-casein-depleted micelles, in a manner somewhat analogous to rennet coagulation, although the mechanism by which the altered micelles are produced is very different.

As would be expected, heating milk at 140°C for an extended period causes very significant chemical and physical changes in milk, of which the following are probably the most significant:

1. Decrease in pH. After heating at 140°C for 20 min, the pH of milk has decreased to about 5.8 due to acid production from pyrolysis of lactose, precipitation of soluble calcium phosphate as $Ca_3(PO_4)_2$, with the release of H^+, and dephosphorylation of casein with subsequent precipitation of the liberated phosphate as $Ca_3(PO_4)_2$ with the release of H^+. The heat-induced precipitation of $Ca_3(PO_4)_2$ is partially reversible on cooling so that the actual pH of milk at 140°C at the point of coagulation is much lower than the measured value and is probably below 5.0.

2. Precipitation of soluble calcium phosphate as $Ca_3(PO_4)_2$ with the release of H^+. After heating at 140°C for 5–10 min, most (>90%) of the soluble phosphate has been precipitated.
3. Dephosphorylation of casein, which follows first-order kinetics. After heating at 140°C for 60 min, >90% of the casein phosphate groups have been hydrolysed.
4. Maillard browning, which occurs rapidly at 140°C. Since Maillard browning involves blocking of the ε-amino group of proteins with a concomitant reduction in protein charge, it would be expected that Maillard browning would reduce HCT, but in fact the Maillard reaction appears to increase heat stability, possibly owing to the formation of low molecular weight carbonyls.
5. Hydrolysis of caseins. During heating at 140°C there is a considerable increase in non-protein N (12% TCA-soluble), apparently following zero-order kinetics. κ-Casein appears to be particularly sensitive to heating and about 25% of the *N*-acetylneuraminic acid (a constituent of κ-casein) is soluble in 12% TCA at the point of coagulation.
6. Cross-linking of proteins. Covalent cross-linking of caseins is evident (by gel electrophoresis) after even 2 min at 140°C and it is not possible to resolve the heat-coagulated caseins by urea– or SDS–PAGE.
7. Denaturation of whey proteins. Whey proteins are denatured very rapidly at 140°C; as discussed in section 9.6.3, the denatured proteins associate with the casein micelles, via sulphydryl–disulphide interactions with κ-casein, and probably with α_{s2}-casein, at pH values below 6.7. The whey proteins can be seen in electron photomicrographs as appendages on the casein micelles.
8. Association and shattering of micelles. Electron microscopy shows that the casein micelles aggregate initially, then disintegrate and finally aggregate into a three-dimensional network.
9. Changes in hydration. As would be expected from many of the changes discussed above, the hydration of the casein micelles decreases with the duration of heating at 140°C. The decrease appears to be due mainly to the fall in pH – if samples are adjusted to pH 6.7 after heating, there is an apparent increase in hydration on heating.
10. Surface (zeta) potential. It is not possible to measure the zeta potential of casein micelles at the assay temperature but measurements on heated micelles after cooling suggest no change in zeta potential, which is rather surprising since many of the changes discussed above would be expected to reduce surface charge.

All the heat-induced changes discussed would be expected to cause major alterations in the casein micelles, but the most significant change with respect to heat coagulation appears to be the decrease in pH – if the pH is readjusted occasionally to pH 6.7, milk can be heated for several hours at 140°C without coagulation. The stabilizing effect of urea is, at least partially,

due to the heat-induced formation of NH_3 which reduces or delays the fall in pH; however, other mechanisms for the stabilizing effect of urea have been proposed.

9.7.1 Effect of processing operations on heat stability

Concentration. Concentration by thermal evaporation markedly reduces the heat stability of milk, e.g. concentrated skim milk containing about 18% total solids coagulates in roughly 10 min at 130°C. The stability of the concentrate is strongly affected by pH, with a maximum at around pH 6.6, but stability remains low at all pH values above about 6.8 (Figure 9.20). Concentration by ultrafiltration has a much smaller effect on HCT than thermal evaporation, due to a lower concentration of soluble salts in the retentate.

Homogenization. Homogenization of skim milk has no effect on HCT but it destabilizes whole milk, the extent of destabilization increasing with fat content and the severity of homogenization (Figure 9.21). Destabilization probably occurs because the fat globules formed on homogenization are stabilized by casein and consequently they behave as 'casein micelles', in effect increasing the concentration of coagulable material.

Forewarming (preheating). Heating an unconcentrated milk, especially at 90°C × 10 min, before a heat stability assay, reduces its heat stability,

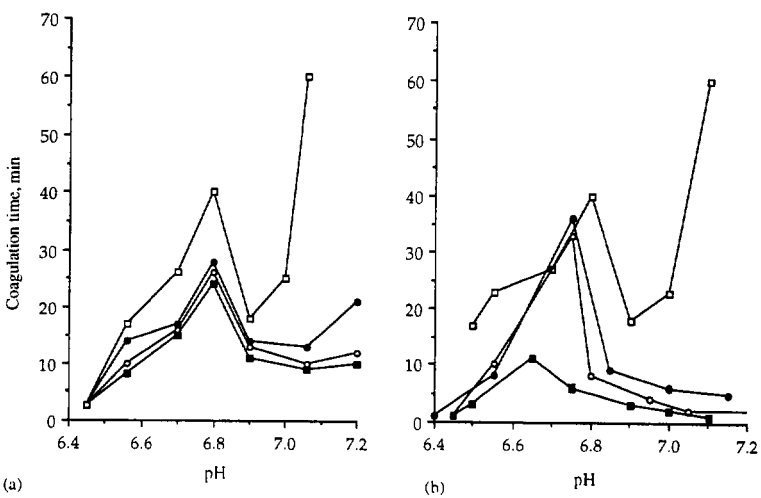


Figure 9.20 Effect of total solids (TS) content on the heat stability at 130°C of skim milk □, 9.3% TS; ●, 12.0% TS; ○, 15.0% TS; ■, 18.4% TS. (a) Concentrated by ultrafiltration, (b) concentrated by evaporation (from Sweetsur and Muir, 1980).

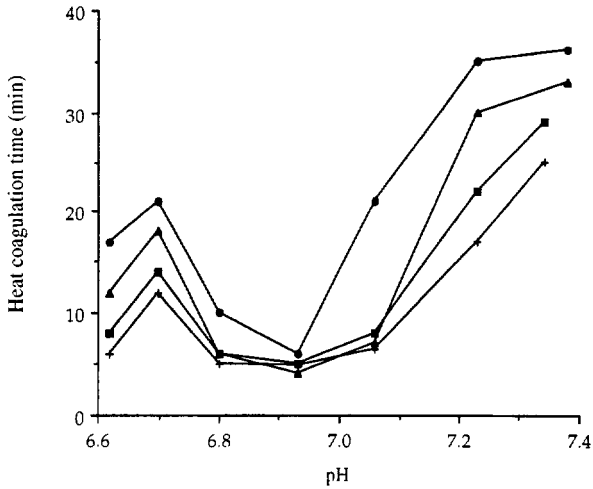


Figure 9.21 Effect of pressure (Rannie homogenizer) on the heat coagulation time (at 140°C) of milk, unhomogenized (●) or homogenized at 3.5 MPa; (▲); 10.4 MPa (■) or 20.7/3.5 MPa (+) (from Sweetsur and Muir, 1983).

mainly by shifting its natural pH; maximum heat stability is affected only slightly or not at all. However, if milk is preheated before concentration, the heat stability of the concentrate is increased. Various preheating conditions are used, e.g. 90°C × 10 min, 120°C × 2 min or 140°C × 5 s; the last is particularly effective but is not widely used commercially. The stabilizing effect is probably due to the fact that the heat-induced changes discussed previously are less detrimental if they occur prior to concentration rather than in concentrated milk which is inherently less stable.

Additives. Orthophosphates, and less frequently citrates, have long been used commercially to increase the stability of concentrated milk. The mechanism was believed to involve Ca-chelation but pH adjustments may be the principal mechanism.

Numerous compounds increase heat stability (e.g. various carbonyls, including diacetyl, and ionic detergents) but few are permitted additives. Although added urea has a major effect on the stability of unconcentrated milk, it does not stabilize concentrated milks, although it does increase the effectiveness of carbonyls.

9.8 Effect of heat treatment on rennet coagulation of milk and related properties

The primary step in the manufacture of most cheese varieties and rennet casein involves coagulation of the casein micelles to form a gel. Coagulation

involves two steps (phases), the first of which involves enzymatically hydrolysing the micelle-stabilizing protein, κ-casein, by selected proteinases, referred to as rennets. The second step of coagulation involves coagulation of rennet-altered micelles by Ca^{2+} above 20°C (Chapter 10).

The rate of rennet coagulation is affected by many compositional factors, including the concentrations of Ca^{2+}, casein and colloidal calcium phosphate and pH. Coagulation is adversely affected by heat treatment of the milk at temperatures above about 70°C due to interaction of denatured β-lg (and α-la) with κ-casein. The primary and, especially, the secondary phases of rennet coagulation are adversely affected by the interaction and, if the heat treatment is sufficiently severe (e.g. 80°C × 5–10 min), the milk does not coagulate on renneting. The effect on the primary phase is presumably due to blockage of the rennet-susceptible bond of κ-casein following interaction with β-lg. The adverse effect of heating on the second phase arises because the whey protein-coated micelles are unable to interact properly because the aggregation sites, which are unknown, are blocked.

The adverse effects of heat treatment on the rennetability of milk can be offset by acidifying or acidifying–reneutralizing the heated milk or supplementing it with Ca^{2+}. The mechanism by which acidification offsets the adverse effects of heating is not known but may involve changes in Ca^{2+} concentration.

The strength of the rennet-induced gel is also adversely affected by heat treatment of the milk, again presumably because the whey protein-coated micelles are unable to participate properly in the gel network. Gels from severely heat-treated milk have poor syneresis properties, resulting in high-moisture cheese which does not ripen properly. Syneresis is undesirable in fermented milks, e.g. yoghurt, the milk for which is severely heat-treated (e.g. 90°C × 10 min) to reduce the risk of syneresis.

9.9 Age gelation of sterilized milk

Two main problems limit the shelf-life of UHT sterilized milks: off-flavour development and gelation. Age gelation, which also occurs occasionally with in-container sterilized concentrated milks, is not related to the heat stability of the milk (provided that the product withstands the sterilization process) but the heat treatment does have a significant influence on gelation, e.g. indirectly heated UHT milk is more stable to age gelation than the directly heated product (the former is the more severe heat treatment). Plasmin may be responsible for the gelation of unconcentrated UHT milk produced from good-quality milk, while proteinases from psychrotrophs are probably responsible if the raw milk was of poor quality. It is possible that physicochemical phenomena are also involved, e.g. interaction between whey proteins and casein micelles.

Table 9.3 Substances making a strong contribution to the flavour of indirectly heated UHT milk, those contributing to differences in flavour of milk heat-treated in different ways, and those used in a synthetic UHT flavour preparation (from Manning and Nursten, 1987)

	UHT-i[a]	UHT-i-LP[b]	UHT-i-UHT-d[c]	Synthetic UHT flavour[d] (mg per kg LP)
Dimethyl sulphide	+	0	1	
3-Methylbutanal	+	1	1	
2-Methylbutanal	+	0	1	
2-Methyl-1-propanethiol	+	1	1	0.008
Pentanal	+	1	1	
3-Hexanone	+			
Hexanal	+	1	1	
2-Heptanone	+	4	2	0.40
Styrene	+			
Z-4-Heptenal[e]	+	1	0	
Heptanal	+			
2-Acetylfuran	+			
Dimethyl trisulphide	+	2	0	
Cyanobenzene	+			
1-Heptanol	+			
1-Octen-3-one[e]	+			
Octanal	+	1	1	
p-Cymene	+			
Phenol	+			
Indene	+			
2-Ethyl-1-hexanol	+			
Benzyl alcohol	+			
Unknown	+			
Acetophenone	+	1	0	
1-Octanol	+			
2-Nonanone	+	4	2	0.21
Nonanal	+			
p-Cresol	+			
m-Cresol	+			
E-2,*Z*-6-Nonadienal	+			
E-2-Nonenal	+			
3-Methylindene	+			
Methylindene	+			
Ethyldimethylbenzene	+			
Decanal	+			
Tetraethylthiourea	+			
Benzothiazole	+	1	0	0.005
γ-Octalactone	+	1	0	0.025
2,3,5-Trimethylanisole	+			
δ-Octalactone	+	1	0	
1-Decanol	+	1	1	
2-Undecanone	+	2	1	0.18
2-Methylnaphthalene	+			
Indole	+			
δ-Decalactone	+	1	0	0.650
Hydrogen sulphide		2	1	0.03
Diacetyl		2	1	0.005
Dimethyl disulphide		2	1	0.002
2-Hexanone		2	1	

Table 9.3 (*Continued*)

	UHT-i[a]	UHT-i-LP[b]	UHT-i-UHT-d[c]	Synthetic UHT flavour[d] (mg per kg LP)
γ-Dodecalactone		2	1	0.025
δ-Dodecalactone		2	1	0.1
Methanethiol		1	1	0.002
2-Pentanone		1	1	0.29
Methyl isothiocyanate		1	1	0.01
Ethyl isothiocyanate		1	1	0.01
Furfural		1	1	
Benzaldehyde		1	0	
2-Octanone		1	0	
Naphthalene		1	0	
γ-Decalactone		1	0	
2-Tridecanone		1	0	
Acetaldehyde		−1	0	
1-Cyano-4-pentene		−1	0	
2-Methyl-1-butanol		−1	1	
Ethyl butyrate		−1	0	
3-Buten-1-yl isothiocyanate		−1	0	
E-2,*E*-4-nonadienal		−1	0	
2,4-Dithiapentane			1	
Maltol				10.00

[a]Indirectly heated UHT milk; + indicates a component that makes a strong contribution to the flavour. In addition to the components listed, a further 12 unknowns made strong contributions.
[b]Components contributing to a difference in flavour between indirectly heated UHT milk and low temperature pasteurized (LP) milk. Scale for difference: 1, slight; 2, moderate; 3, strong; 4, very strong.
[c]Components contributing to a difference in flavour between indirectly and directly heated UHT milks. Scale for difference as in [b].
[d]Composition of synthetic UHT flavour.
[e]Tentative identification.

In the case of concentrated UHT milks, physicochemical effects appear to predominate, although proteolysis also occurs, e.g. the propensity of UHT concentrated milk reconstituted from high-heat milk powder to age gelation is less than those from medium- or low-heat powders, although the formation of sediment is greatest in the concentrate prepared from the high-heat powder (see Harwalkar, 1992).

9.10 Heat-induced changes in flavour of milk

Flavour is a very important attribute of all foods; heating/cooking makes a major contribution to flavour, both positively and negatively. Good-quality fresh liquid milk products are expected to have a clean, sweetish taste and essentially no aroma; any departure therefrom can be considered as an

off-flavour. Heat treatments have a major impact on the flavour/aroma of dairy foods, either positively or negatively.

On the positive side, thermization and minimum pasteurization should not cause the formation of undesirable flavours and aromas and should, in fact, result in improved flavour by reducing bacterial growth and enzymatic activity, e.g. lipolysis. If accompanied by vacuum treatment (vacreation), pasteurization removes indigenous off-flavours, i.e. those arising from the cow's metabolism or from feed, thereby improving the organoleptic qualities of milk.

Also on the positive side, severe heat treatment of cream improves the oxidative stability of butter produced therefrom due to the exposure of antioxidant sulphydryl groups. As discussed in section 9.2.2, lactones formed from hydroxyacids are major contributors to the desirable cooking quality of milk fats but contribute to off-flavours in other heated products, e.g. milk powders.

UHT processing causes substantial deterioration in the organoleptic quality of milk. Freshly processed UHT milk is described as 'cooked' and 'cabbagy', but the intensity of these flavours decreases during storage, giving maximum flavour acceptability after a few days. These off-flavours are due to the formation of sulphur compounds from the denatured whey proteins, as discussed in section 9.6.3. After this period of maximum acceptability, quality deteriorates, the milk being described as stale. At least 400 volatiles have been detected in UHT milk, about 50 of which (Table 9.3) are considered to make a significant contribution to flavour (Manning and Nursten, 1987). The shelf-life of UHT milk is usually limited by gelation and/or bitterness, both of which are due to proteolysis, as discussed in section 9.6.1.

Since sulphur compounds are important in the off-flavour of UHT milk, attempts to improve its flavour have focused on reducing the concentration of these, e.g. by adding thiosulphonates, thiosulphates or cystine (which react with mercaptans) or sulphydryl oxidase, an indigenous milk enzyme (which oxidizes sulphydryls to disulphides; Chapter 8).

The products of Maillard browning have a significant negative impact on the flavour of heated milk products, especially in-container sterilized milks and milk powders.

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